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DNA SYNTHESIS AND THE ANNUAL SPERMATOGENIC CYCLE IN INDIVIDUALS OF THE SEA STAR *PATIRIA MINIATA*

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ABSTRACT

Male individuals of the sea star *Patiria miniata* have an annual spermatogenic cycle. This cycle was first suggested by gonad-index and histological analyses for a population of *P. miniata* in the field. The cycle was confirmed by tritiated-thymidine autoradiography of testes surgically removed about every 2 months from each of 10 individuals kept in the laboratory. Tritiated-thymidine autoradiography also revealed that the spermatogenic cycle includes a period of quiescence when sperm are not being produced and DNA synthesis is not occurring. Repeated surgical sampling of testes from individuals and autoradiography with tritiated thymidine are potentially useful methods for laboratory studies on the regulation of DNA synthesis and spermatogenesis and on regeneration.

INTRODUCTION

The study of spermatogenesis in asteroids can contribute significantly to our understanding of spermatogenesis in general and the factors regulating it. An annual spermatogenic cycle has already been established for many species of asteroids (*cf.* Shick *et al.*, 1981; Walker, 1982). Recently, several investigators have taken up the question of what exogenous or endogenous factors regulate spermatogenesis and the spermatogenic cycle in asteroids (Delavault and Bruslé, 1970; Kanatani, 1973; Schoenmakers *et al.*, 1976; Kubota *et al.*, 1977; Walker, 1980; Pearse and Eernisse, 1982; Walker and Larochelle, 1984).

Patiria miniata, a gonochoric asterinid asteroid, can be a valuable animal for studies on spermatogenesis. In central and northern California it is abundant in protected intertidal rocky areas and subtidal kelp forests (Ricketts *et al.*, 1968; Pearse and Lowry, 1974; Gerard, 1976; Feder, 1980). It is extremely hardy and therefore suitable for laboratory studies, including those involving surgery and organ culture (*cf.* Davis, 1982). Previous studies indicate that *P. miniata* probably has an annual gametogenic cycle, although the cycles of different individuals are not very synchronous, and sperm and ripe eggs can be obtained from at least some animals at almost any time of the year (Farmanfarmaian *et al.*, 1958; Lawrence, 1965; MacGinitie and MacGinitie, 1968; Ricketts *et al.*, 1968; Nimitz, 1971, 1976; Gerard, 1976).

One of the questions addressed in the present study is whether DNA synthesis in germinal cells occurs throughout an annual spermatogenic cycle or is restricted to particular periods. These periods may be defined by the calendar or by other events in the cycle. Since the process of spermatogenesis involves many cell divisions and these are preceded by DNA synthesis, the starting and stopping of DNA synthesis in spermatogonia or spermatocytes may indicate the location and timing of factors regulating spermatogenesis. Autoradiography with tritiated thymidine, the technique used

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in this study, has previously been used for observations of DNA synthesis in testes at different points in the spermatogenic cycle in the sea urchin *Strongylocentrotus purpuratus* (Holland and Giese, 1965), the sea star *Asterias rubens* (Van der Plas *et al.,* 1983), and the brittle star *Amphipholis kochii* (Yamashita and Iwata, 1983).

In the present study DNA synthesis during the annual spermatogenic cycle was followed both for a population of animals in the field and for a group of individuals in the laboratory. The lab study was to determine whether the rather asynchronous individuals from the field population each have a cycle of spermatogenesis and DNA synthesis. Delavault (1963) repeatedly removed gonads surgically from individuals of the sea star *Asterina gibbosa.* It appeared that this technique would permit following the reproductive cycle in individuals of *Patiria miniata,* which has a body wall soft enough to be easily cut.

Materials and Methods

Animal collection and maintenance

Individuals of *Patiria miniata* were collected subtidally by SCUBA from a kelp forest in the Hopkins Marine Life Refuge, at Point Cabrillo, on Monterey Bay, California. Animals with the longest arm between 60 and 80 mm long were collected approximately every two months for two and a half years (Fig. 1). These animals generally weighed between 50 and 120 g. Animals in this size range were used because in preliminary collections I found that in such animals there was no apparent trend of gonadal or pyloric cecal size changing with body size, while in smaller animals there were such trends (Davis, 1982). Sea stars that were not dissected at Hopkins Marine Station the day of collection were kept at Long Marine Laboratory of the University of California at Santa Cruz. In the laboratory the specimens were kept in tanks with fresh-flowing seawater in a room with fluorescent lights (G. E. F40D Daylight) set to come on at local sunrise and go off at local sunset. The temperatures of the seawater in the lab and in the field were indistinguishable. The animals were fed kelp, *Macrocystis pyrifera,* and smashed mussels, *Mytilus californianus.*

Dissection of field animals

For the study of the reproductive cycle in a population, at least 10 males from each of the collections were dissected within a week of collection (Fig. 1). After an animal was weighed and dissected, the following were recorded: wet weight of the 10 gonads; wet weight of the 10 pyloric ceca; and sex, determined from color of gonad (white in males or orange in females) and/or from the presence of sperm or oocytes in a squash. From these weights the gonad index (GI) and pyloric cecum index (PCI) were calculated, each defined as the percent of the body weight comprised by the gonads or pyloric ceca, respectively. Data for females are presented in Davis (1982).

Testes from the males collected from February 1978 through February 1979 were fixed in seawater-Bouin's for histological analysis. Testes from males collected from April 1979 through July 1980 were incubated in tritiated thymidine for autoradiographic analysis (see below) (Fig. 1).

Surgical removal of testes from laboratory animals

For the study of the reproductive cycle in individuals, 20 Point Cabrillo males collected 18 April 1979 were prepared 23 April. *P. miniata* has 10 gonads, two per interradius. For an animal of unknown sex, I located in one interradius the medial

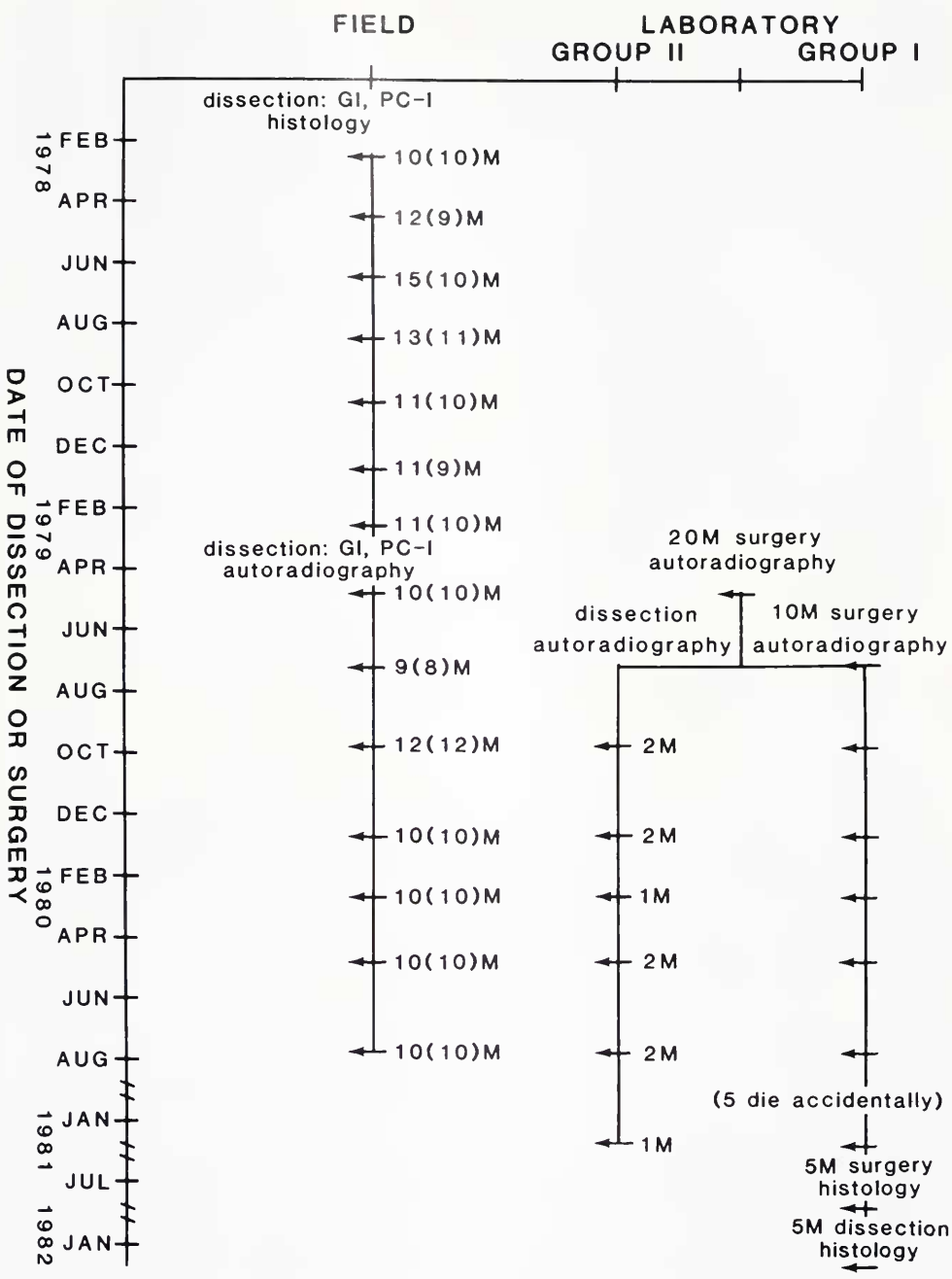

FIGURE 1. Design of the studies of the reproductive cycle in *Patiria miniata*. Animals were either collected from the field approximately every two months, or were collected in April 1979 and then kept in the laboratory. Included are the dates that gonads were removed from animals by surgery or dissection; the numbers of animals used; and the methods of analyses of the gonads, *e.g.*, gonad and pyloric cecum indices, histology, autoradiography. When two numbers are shown for the number of males, the first is the number used for the organ indices, the second (in parentheses) is the number also used for histologic or autoradiographic analyses. M = male. GI = gonad index. PC-I = pyloric cecum index.

line of ossicles that indicates the underlying internal pillar and cut a wedge-shaped flap, the apex pointing outwards, about 1 mm to either side of the medial line. The apex was lifted with forceps, while the gonoduct, attached to the underside of the flap, was gently pulled from the animal along with the duct's gonad. The gonad was then sexed; and for each of the first 20 males, the removed testis was incubated in tritiated thymidine, the wedge-shaped flap was folded back into place, and one or more notches was cut into the side of one or more rays to identify the individual. The 20 males were kept together in one tank.

In July 1979 the sea stars in the common tank were divided into 2 groups of 10 males each, the groups equivalent in their animals' sizes and reproductive states. The two groups were designed to examine for any effects of the frequency of surgery on reproductive state. From each of the 10 sea stars in Group I, a testis was surgically removed and analyzed autoradiographically about every two months through July 1980 at about the same time as the sampling of testes from the field animals (Fig. 1). The first five times testes were removed surgically from a particular individual, the testes were taken from a different interradius each time. Five animals died in the fall of 1980 when the air to the tank accidentally went off. From each of the five remaining individuals, testes were removed surgically in January and July 1981; and the animals themselves were dissected in January 1982 (Fig. 1). The 10 males in Group II were not surgically sampled again after 23 April 1979 but were dissected at various times: one or two animals about every two months through July 1980, the tenth animal in January 1981 (Fig. 1). For both Groups I and II, whenever a testis was surgically removed from an interradius from which a testis had been taken at an earlier date, or any time an animal was dissected, any regeneration of previously removed testes was noted.

Histology and autoradiography

Usually one entire testis per animal was used for the incubations in tritiated thymidine. In some sampling periods, however, two or three testes from each animal were used. Comparisons among the multiple testes within an animal, including comparisons between intact testes and testes in pieces, showed no obvious difference in distribution of radioactive label (Davis, 1982).

Each testis was incubated two hours in 5–15 ml of 3 μC/ml of tritiated thymidine in seawater. The incubation flasks were shaken in a water bath cooled by cooling coils hooked up directly to a spigot of the laboratory's seawater system. The temperatures of the seawater in the water bath for incubations from April 1979 through January 1981 ranged from 12.9 to 15.9°C and were always within 0.5°C of the temperature of the seawater in the laboratory's seawater system. After incubation, the tissues were fixed in seawater-Bouin's.

Following fixation, the tissues were dehydrated in an alcohol series, embedded in Paraplast Plus, and sectioned at 7 or 10 μm for histological analysis or 5 μm for autoradiographic analysis. The autoradiographic slides were then dipped in Kodak NTB-2 emulsion and exposed to the emulsion for two to eight weeks. All slides were stained with standard alum hematoxylin (Galigher and Kozloff, 1971) and eosin.

Analysis

To minimize bias in my descriptions of reproductive states, I mixed together the slides from all samples and covered their labels. The labels were uncovered only after all slides were described. While there was some variation in reproductive state between

lobes within a testis, it was not difficult in most cases to assign a reproductive state on the basis of a clear majority of lobes.

Each testis was ranked according to stage in the reproductive cycle. Several criteria were used in this description. These included the following histological characteristics of the germinal epithelium: presence or absence of spermatogenic columns, the types of germinal cells within the columns, the thickness of the spermatogonia-spermatocyte layer relative to the thickness of the spermatid layer, and the overall thickness of the germinal epithelium. The abundance of sperm in the lumen was also noted. Additional criteria were the proportion of spermatogonia and spermatocytes labeled with tritiated thymidine, and the thickness of the labeled cell layer relative to the thickness of the unlabeled cell layer.

On the basis of the above parameters, I rated the reproductive state of each testis with a number from 0.0 to 3.5, where 0 = inactive, 1 = early spermatogenesis, 2 = active spermatogenesis, 3 = ripe. The distinguishing characteristics of the categories are shown in Table I. Testes intermediate between two of these states or with mixtures of lobes differing in state by as much as one whole number were given intermediate numbers. As a 0.0 (inactive) was considered equivalent to a hypothetical 4.0 (spent), testes intermediate between 3.0 and 0.0—that is, partially spawned ones—were rated 3.5.

RESULTS

Reproductive cycle in the Point Cabrillo population

There was considerable individual variability among the gonad and pyloric cecum indices of male *P. miniata* collected over a period of two and a half years (Fig. 2). The mean gonad index differed significantly from month to month (ANOVAs, $P < .01$), but the month in which it was highest and the highest value reached varied from year to year. The eight highest mean gonad indices occurred from December through June, the six lowest from July through December. Thus, Figure 2 suggests that spermatogenesis occurred from early or late fall to early spring, and spawning in late spring or early summer. The mean pyloric cecum index differed significantly from month to month (ANOVAs, $P < .01$), but there were no consistent year-to-year trends (Fig. 2).

Testes of various reproductive states from *P. miniata* are shown in Figure 3A–F. The histology is similar to that in other asteroids (*e.g.*, Pearse, 1965; Walker, 1980).

TABLE I

Characters used to distinguish reproductive states of testes from Patiria miniata

Reprod. state	Columns	Types of cells in g.e.	Proportion of G and C labeled	Relative thicknesses of cell layers	Abundance of sperm in lumen
0.0	No	G	few	– –	generally few
1.0	No	G	many	– –	– –
1.5	Yes	G, C	most	– –	– –
2.0	Yes	G, C, T	most	T < 1/2 g.e.	– –
2.5	Yes	G, C, T	most	T > 1/2 g.e.	– –
3.0	No	G, C, or T	variable	– –	many

G = spermatogonia. C = spermatocytes. T = spermatids. g.e. = germinal epithelium. The "– –" in columns denotes characteristics that were not used to distinguish states.

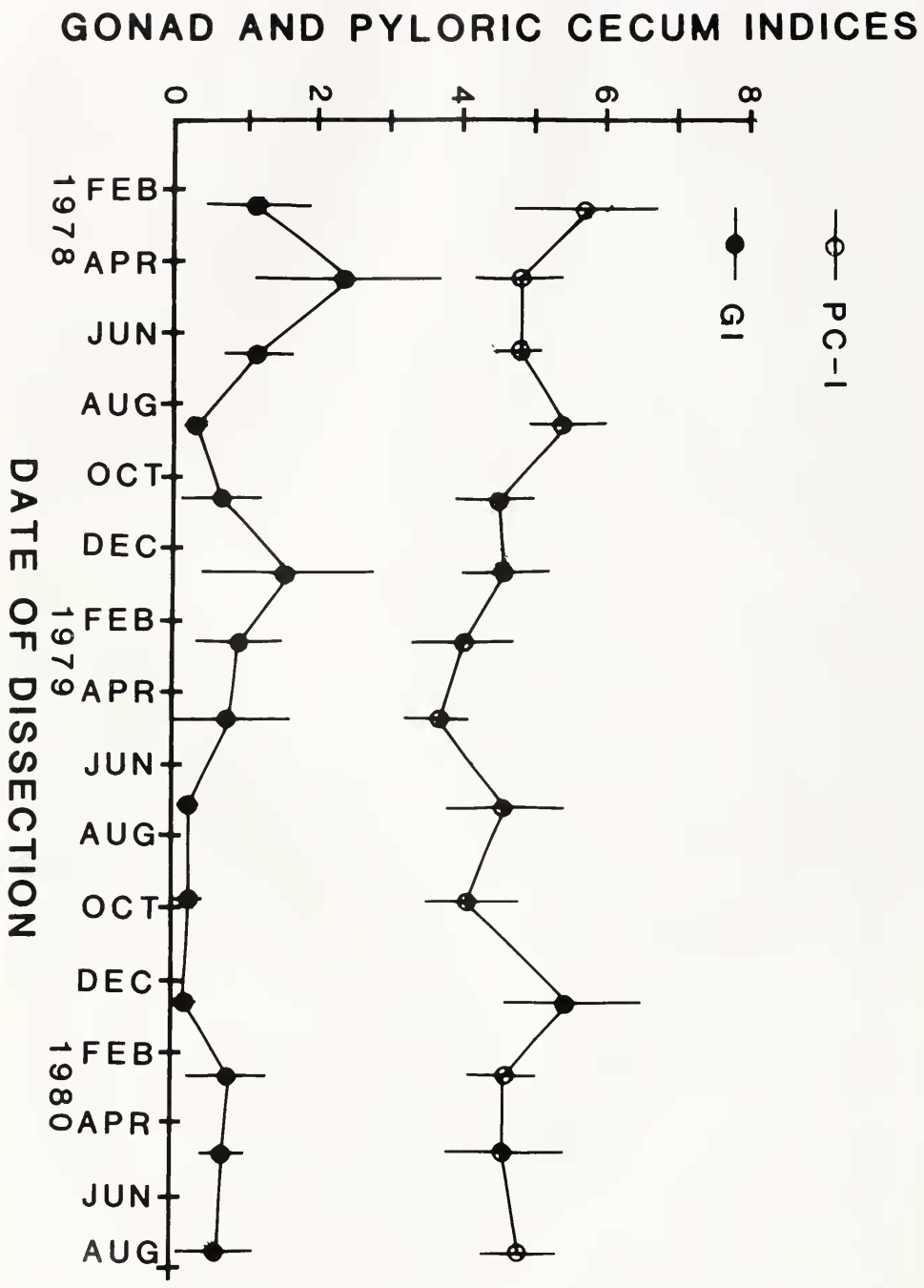

FIGURE 2. Gonad and pyloric cecum indices of *Patiria miniata* collected from the field approximately every two months. Mean ± 95% confidence limits. GI = gonad index. PC-I = pyloric cecum index.

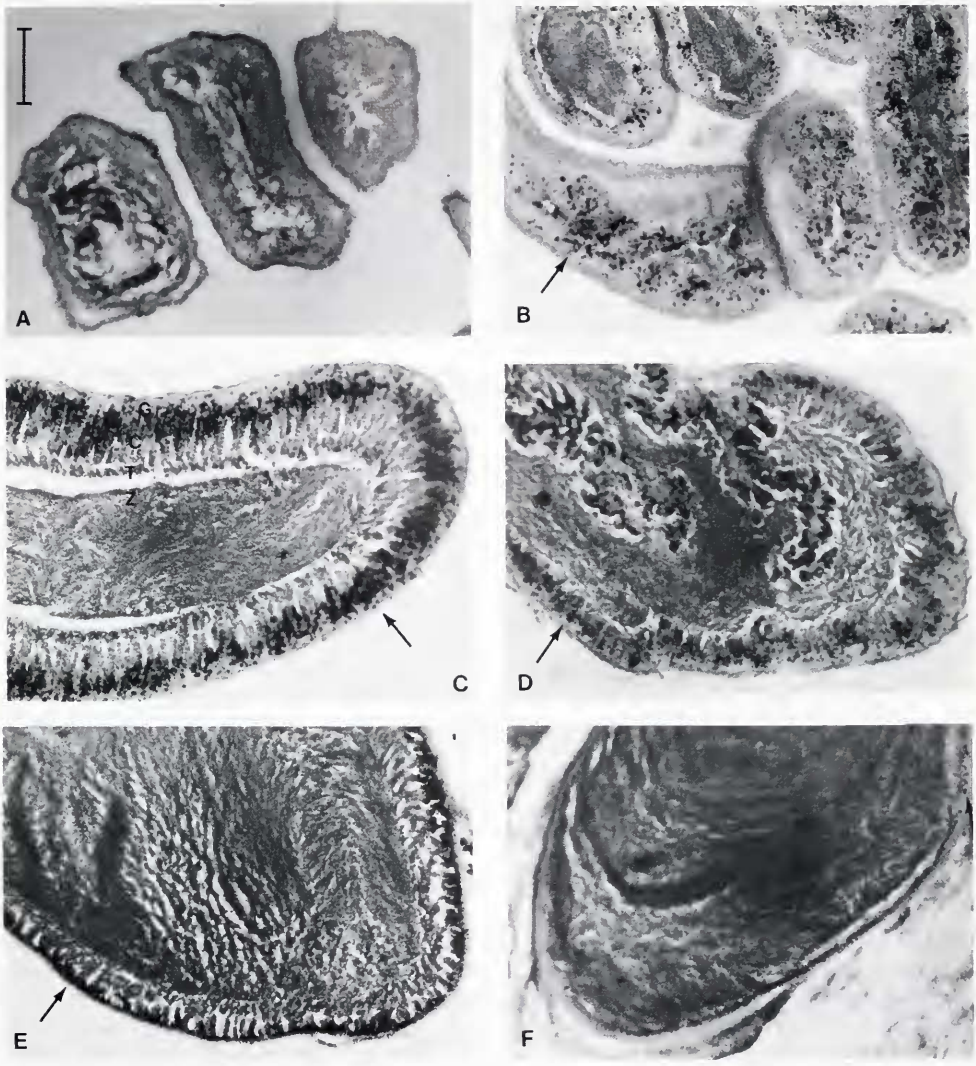

FIGURE 3. Autoradiograms showing various reproductive states of testes from *Patiria miniata*. Arrows indicate areas with silver grains representing DNA synthesis. Scale = 100 μm. A: 0.0 (inactive). B: 1.0 (early spermatogenesis). C: 2.0 (active spermatogenesis). G, C, and T indicate areas of the spermatogonia, spermatocytes, and spermatids, respectively, in the spermatogenic columns of the germinal epithelium. Z indicates sperm in the lumen. D: 2.0 (active spermatogenesis), with branching germinal epithelium. E: 2.5. F: 3.0 (ripe).

A testis consists of lobulated sacs. Around the inner wall of a testicular lobe is the germinal epithelium, containing various types of germinal cells, which may be distinguished from one another on the basis of position, size, appearance of chromatin, and staining intensity. In a testis actively producing sperm (*cf.* Fig. 3C), the germinal epithelium is comprised of columns, with the spermatogonia at the base, followed distally by spermatocytes, then spermatids. In a 2.0 testis, most of the spermatocytes closer to the bases of the columns have radioactive label over them, while fewer of the

spermatocytes closer to the spermatids do (Fig. 3C). Sperm are in the lumen of the testis. Sectioned germinal epithelium in 2.0 testes was frequently seen in the lumen. When I traced it in serial sections of some testes, I found that it joined the germinal epithelium around the inner wall of the testis. The germinal epithelium in the lumen resulted in some cases from the infolding of the hemal space, which surrounds the germinal epithelium in the wall; in other cases it arose from the branching of spermatogenic columns (Fig. 3D).

The reproductive states of the animals sampled from the field approximately every two months from February 1978 through July 1980 are shown in Figure 4. Prior to April 1979 the ratings were based on histology; after April 1979 they were based on both histology and autoradiography. The main consequence of this switch of criteria is that some of the testes rated 0.0 (*e.g.*, in August and October 1978) probably had some DNA synthesis and therefore would have been rated 0.5 to 1.0 with autoradiography. States 1.5 to 2.5 were the most spermatogenically active testes. That is, these had spermatogenic columns and more cells synthesizing DNA than the other states. In testes of states beyond 2.5, the germinal epithelium was nearing the end of the spermatogenic cycle, while in those below 1.5 it was either inactive (0.0) or beginning synthetic activity (1.0) (Fig. 3, Table I).

In general, stage 0.0 predominated in summer, 1.0 in late fall, 2.0 in winter, 2.5 in spring, and 3.0 in late spring. However, the timing of these stages varied from year to year. For example, testes in December 1978 were more advanced than those in December 1979, and testes in April 1979 and 1980 were more advanced than those in April 1978. There was also individual variability; not all animals in a sample were at the same stage of reproductive development (Fig. 4).

Gonad index was not a good indicator of reproductive state except that gonad indices exceeding 0.5 almost always indicated testes of states 2.0–3.0 (Fig. 5). In general, there was no relationship between pyloric cecum index and reproductive state. However, while the mean pyloric cecum index for reproductive state 0.0 was similar to the mean indices for individuals in more developed reproductive states, the lowest indices—three or less—all were associated with state 0.0 (Fig. 5).

Reproductive cycle in the laboratory animals

An annual progression of spermatogenic stages was also observed in the *Patiria* kept in the laboratory (Fig. 4). Furthermore, the cycle could be followed within individuals. Thus, of the 10 sea stars in Group I, 6 were spermatogenically active in September and December 1979 and in February and April 1980; 2 (individuals #9 and #17) lagged behind; 1 (#19) may never have produced sperm during the year; and 1 (#10) clearly did not (Fig. 6). At each sampling period from September 1979 through January 1981, the reproductive states in Group II (the laboratory animals sampled surgically in April 1979 and not again until dissected) were comparable to those in Group I (Fig. 6, Table II). However, the testes of both groups of laboratory animals were clearly more advanced than those in the field animals in September and December 1979, even though there was little difference in July 1979 (Figs. 4, 6; Table II).

From July 1980 through January 1982, four of the five Group I animals that were sampled every six months continued spermatogenesis until the tenth testis was removed from the body, while the fifth animal, individual #17, apparently did not (Fig. 6). These four animals had testes of reproductive states 1.5–2.0 in January 1981 and January 1982, which was in accord with the states of testes taken at that time of year from the lab animals in the previous year and from the field animals in the previous two years (Figs. 4, 6). However, in July 1981, testes from three of the four animals had testes of reproductive states 2.0–2.5 (Fig. 6). This was surprising since the field

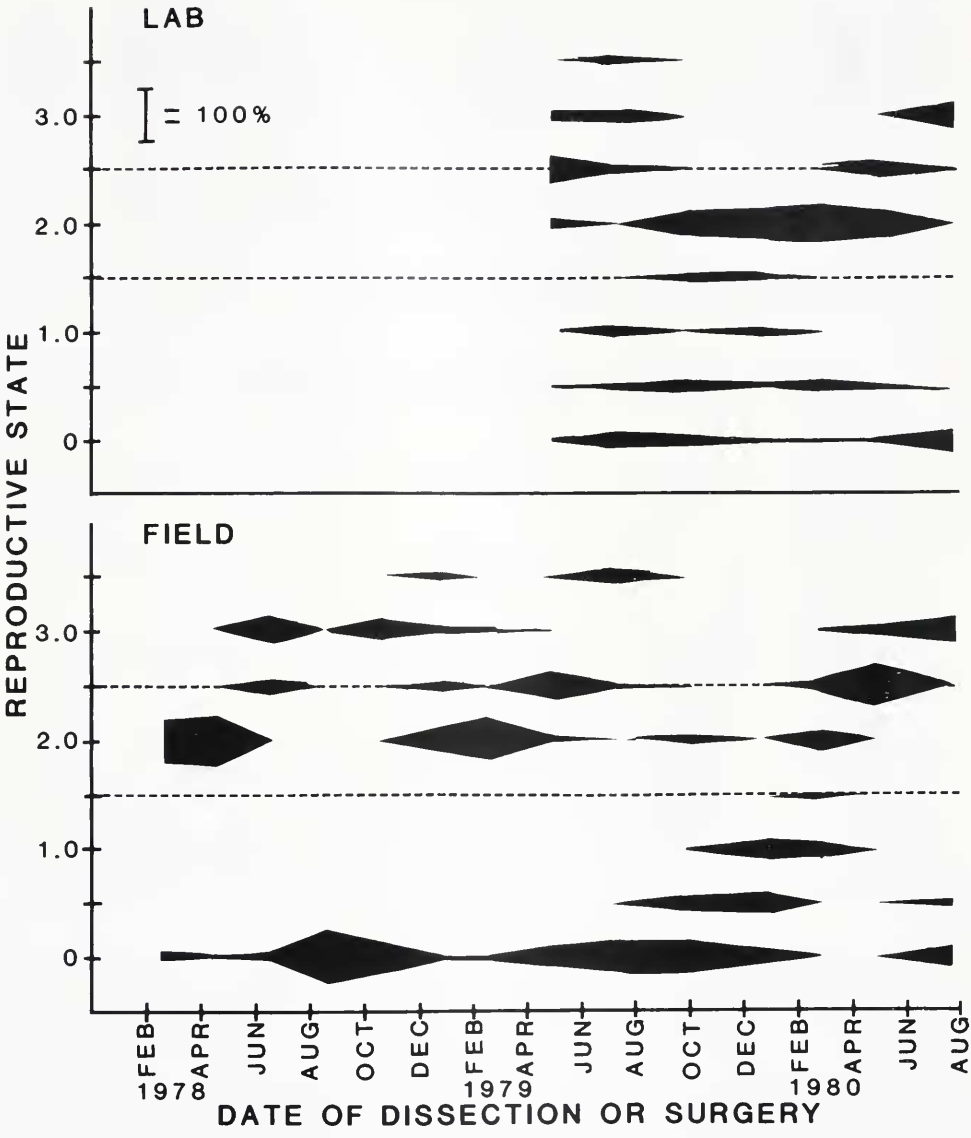

FIGURE 4. Reproductive states of testes from *Patiria miniata*. The testes were taken by dissection from field animals or by surgery from Group I laboratory animals. The width of the polygons represents the percent of the individuals in a sample that had testes of the reproductive states indicated. The scale indicates the width corresponding to 100%. Reproductive states from 1.5 through 2.5, indicated by the area between the dashed lines, represent the most spermatogenically active testes.

and laboratory data from the previous three summers had indicated that spermatogenic columns were usually absent from testes in July (Fig. 4).

Regeneration of gonads

The data on regeneration (or lack thereof) for the testes surgically removed from the lab animals were inconsistent. When the 10 Group II animals were dissected at

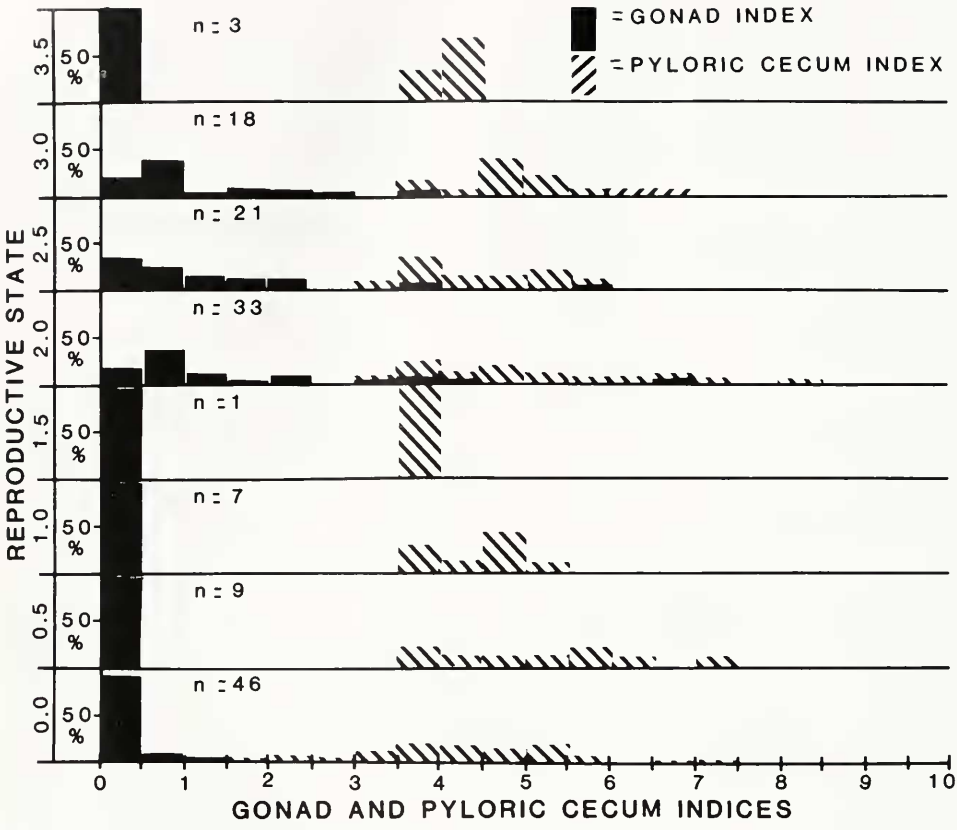

FIGURE 5. Gonad and pyloric cecum indices *versus* reproductive state for male *Patiria miniata* collected from the field between February 1978 and July 1980. The height of a column represents the percent of the individuals with the reproductive state indicated that had organ indices in the increments indicated.

various times between September 1979 and January 1981, only twice, once each in April and July 1980, was some testis found at the position from which a testis had been removed in April 1979. In Group I animals, when the second of the two testes at an interradius was surgically removed, there was, in 23 of 25 observations, no testis at the position from which the other testis at that interradius had been removed a year or more earlier. When the five Group I animals were dissected in January 1982, three of the animals clearly had some testis at one to six positions from which testes had previously been removed; but the number of presumably regenerated testes did not appear related to the time since removal of the testes.

Effects of surgery

Surgery appeared to have little adverse effect upon the behavior of the sea stars. Immediately after surgery, the animals were put on their backs in the tanks to see whether they could right themselves normally. They did so readily. They also crawled up the sides of the tank as usual. Smashed mussels were not added to the tank the week or two after surgery; but when they were, they were quickly eaten.

How fast the wounds healed depended upon how many of the edges of the wedge-

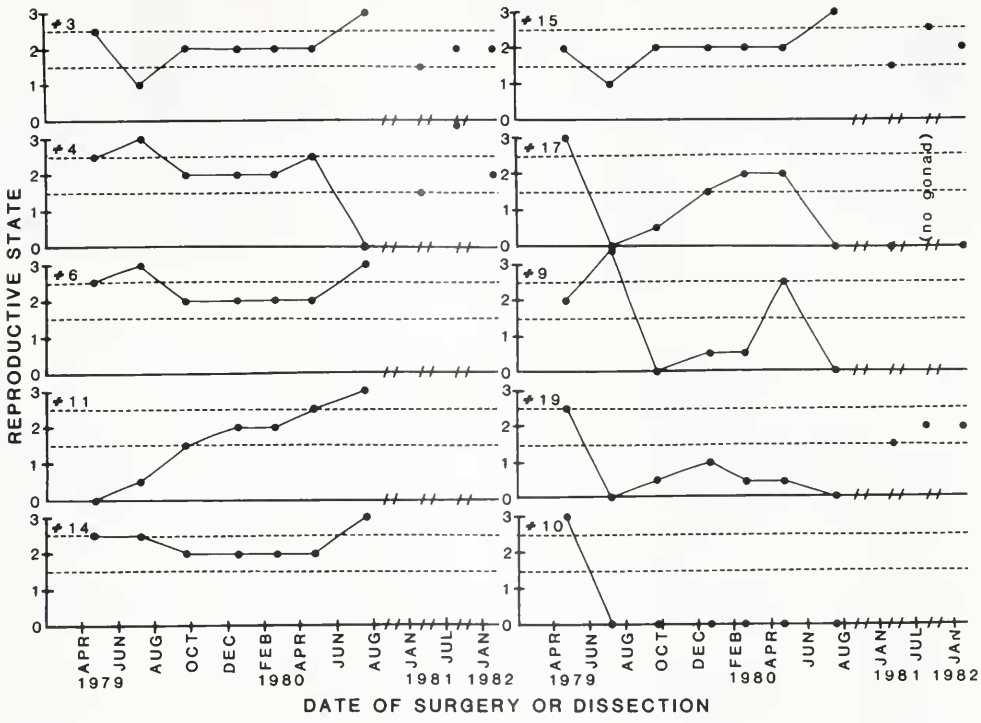

FIGURE 6. Reproductive states of testes from Group I individuals of *Patiria miniata* kept in the laboratory. Each animal was surgically sampled approximately every two months from April 1979 through July 1980, then every six months through dissection in January 1982. Each graph represents the testes from an individual whose identifying number follows the "#" symbol. (Five animals died accidentally in the fall of 1980.) Reproductive states from 1.5 through 2.5, indicated by the area between the dashed lines, represent the most spermatogenically active testes.

shaped flap contacted the edges of body wall surrounding the hole. If all edges of the flap made contact, the wound was often completely sealed within a week and sometimes difficult to see in two to three weeks. After the first surgical sampling in April 1979,

TABLE II

Reproductive states of testes from Group II Patiria miniata—*laboratory-maintained animals, each sampled surgically in April 1979, then sampled again when dissected*

Animal number	Reproductive state in April 1979	Month and year dissected	Reprod. state when dissected
1	2.5	Sep 79	2.0
5	2.5	Sep 79	2.0
2	0.0	Dec 79	2.0
7	2.5	Dec 79	2.0
12	2.5	Feb 80	2.0
8	2.5	Apr 80	2.0
13	2.5	Apr 80	2.5
16	0.0	Jul 80	1.0
18	3.0	Jul 80	3.0
20	2.5	Jan 81	2.0

no pyloric cecum hung out the surgical holes. However, later in the experiment, some pyloric cecum often would hang out after surgery, apparently because the ceca were larger then than at the beginning of the experiment. As a result, the wall flap was less likely to fit completely into place. When this post-surgical misfit occurred, complete sealing took longer; but the seal was always complete before the next sampling two months later.

Occasionally, the edges of the wedge-shaped patch of body wall became slightly necrotic, as evidenced by white puffiness, and in a very few cases this necrosis spread to areas of the body wall surrounding the flap. The development of inflammation in sea stars in response to injury has been discussed by Bang (1982).

Discussion

Spermatogenic cycle in individual Patiria miniata

The results of the present study demonstrate an annual spermatogenic cycle in individual *P. miniata.* This cycle was first suggested by gonad-index and histological analyses for a population of *P. miniata* in the field. The cycle was confirmed by autoradiographic analyses of testes surgically removed from individual animals kept in the laboratory.

Mean gonad indices determined approximately every two months for male *P. miniata* collected from the Point Cabrillo kelp forest suggested that spermatogenesis occurs primarily in winter, and spawning in late spring and early summer. Individuals are quite asynchronous. These conclusions from the present study are in agreement with those of previous workers on *P. miniata,* including the two-year study by Gerard (1976) in the same subtidal study area as the present one; the two-year study in an intertidal area in Tomales Bay by Nimitz (1971); and the one-year studies in intertidal areas near Point Cabrillo by Farmanfarmaian *et al.* (1958) and Lawrence (1965).

My histological studies of the testes from *Patiria miniata* corroborated the impression from the measurements of gonad index that spermatogenesis occurs from fall through spring. The seasonal spermatogenic cycle was evident both in testes taken every two months from animals collected from the field, and in testes of animals kept in the laboratory. Nimitz (1976) reported similar results for the cycle in *P. miniata* in the field. In the present study, good criteria for characterizing the reproductive state of a testis were the presence or absence of spermatogenic columns and the proportions of cell types present in the columns. Measurements of the thickness of the germinal epithelium in *P. miniata* were not useful because of the variability within a testis and often within a lobe. Shick *et al.* (1981) reached the same conclusions about the thickness of the germinal epithelium in testes of *Ctenodiscus crispatus.*

The annual progression of reproductive stages determined from the sampling of testes from seasonal collections of *P. miniata* from the field was confirmed by the autoradiographic analyses of testes surgically sampled from individuals kept in the laboratory. The timing of the onset of spermatogenesis in laboratory animals varied somewhat with the individual, and one or two of the animals did not produce sperm at all (*cf.* Fig. 6). Unfortunately, these two animals died in a water-supply accident in the fall of 1980, so it was not possible to determine whether their gonads would have developed the following year. However, another animal kept in the laboratory as part of a different study did not undergo gametogenesis its first year in the lab even though it appeared to be sexually mature, but did the second year. That animals may sometimes skip a gametogenic cycle suggests that occasional apparently sexually mature winter animals with spermatogenically inactive testes (*cf.* Fig. 4) may be skipping the spermatogenic cycle that year, rather than spawning several months out of synchrony with the rest of the population.

Most of the laboratory animals began spermatogenesis more than two months before those in the field. The reasons for this are not clear but may include any of the following: (1) the animals in the laboratory may have spawned earlier than those in the field, and consequently began the next cycle earlier. (2) The surgical removal of testes from the laboratory animals in April and July 1979 may have caused the remaining testes to develop precociously by September and December 1979, whether due to compensation for missing gonads or release from some inhibition. (3) The environment of the laboratory animals may have differed from that of the field animals in such factors as temperature or the quality or quantity of food.

DNA synthesis during the spermatogenic cycle

The results of the present study also show that germinal cells appear to have a clearly delineated quiescent period in which no sperm are produced and no DNA synthesis occurs. Testes without spermatogenic columns could be divided into two states based upon the absence or presence of DNA synthesis in the germinal epithelium (states 0.0 and 1.0, respectively). Walker (1980) reported that somatic cells with $\Delta 5$-3β-hydroxysteroid dehydrogenase activity appeared in the testicular lumen of *Asterias vulgaris* just before spermatogenic columns formed. Perhaps these somatic cells are associated with the initiation of DNA synthesis in germinal cells.

DNA synthesis was always occurring in all of the spermatogenic columns in the 2.0 testis in *P. miniata*. Radioactive label, indicating DNA synthesis, was generally seen over most spermatogonia, most spermatocytes near the bases of the columns, and fewer but many spermatocytes near the tips of the columns. This distribution of label has also been seen in testes of *Asterias vulgaris* (Walker, 1980), *A. rubens* (Van der Plas *et al.*, 1983), and *Leptasterias pusilla* (Smith, 1971). Walker (1980) found that germinal cells move from the bases toward the tips of the columns during spermatogenesis. Therefore, unlabeled spermatocytes nearer the spermatids are presumably past DNA synthesis.

In the germinal epithelium of stage 2.5 testes in the present study, the thickness of the spermatid layer was greater than that of the spermatogonia-spermatocyte layer. This suggests that, as described by Walker (1980) for *A. vulgaris*, mitoses in the spermatogonia were occurring at a slower rate than were meioses in the spermatocytes. Nevertheless, in *P. miniata* DNA synthesis continued in both the spermatogonia and spermatocytes as the columns were degrading in 2.5 testes. DNA synthesis also occurred in some cells in the germinal epithelium in 3.0 testes, after the columns were gone. Whether DNA synthesis was followed by cell division is not known.

Regeneration of testes

In the present study a variable degree of removal of the genital system along with the gonoduct and testis was probably responsible for the observed variable regeneration of the gonad. Hauenschild (1954) found that the gonads removed from the asterinid *Asterina gibbosa* did not regenerate, while Lender and Huet (1962a, b) and Huet (1965) reported that they did. Okada (1979) found for the sea urchin *Hemicentrotus pulcherrimus* that the degree of regeneration of a removed gonad depended upon the extent of the removal of the gonoduct and blood vessel [= hemal system] to the gonad.

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